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R17526/CMM/RMC

2. Patent application number
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9606961.2

02 APR 1996

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Gwyneth Jane Farrar
The Ocular Genetics Unit
Biotechnology Institute
Genetics Department
Trinity College Dublin
Dublin 2
IRELAND
United Kingdom

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

"Genetic Strategy III"

5. Name of your agent *(if you have one)*

Murgitroyd & Company

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

373 Scotland Street
GLASGOW
G5 8QA

Patents ADP number *(if you know it)*

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1 A

SPECIFICATION

TITLE

Genetic Strategy

FIELD

This invention relates to ..

The present invention relates to a strategy of gene therapy for dominantly acting mutations. The invention achieves suppression of a disease allele using suppression strategies which do not target the disease mutation specifically but instead can be targeted towards a broad range of sequences in a particular gene. A particular embodiment of the invention is the use of suppression strategies to target either the disease or normal alleles alone or to target the disease and normal alleles and the use of the wobble hypothesis to enable continued expression of a reintroduced or replacement normal or beneficial allele. The reintroduced normal gene will have nucleotide changes from the endogenous wild type gene but will code for identical amino acids as the endogenous wild type gene. The strategy circumvents the need for a specific therapy for every mutation in a given gene. In addition the invention allows greater flexibility in choice of target sequences for suppression of a disease allele. The invention also relates to a medicament or medicaments for use in suppressing a disease allele which is present in a genome of one or more individuals or animals.

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HISTORY

In this field it is already known earlier...

Antisense and ribozyme therapies have been used previously to achieve specific suppression of gene expression. For example, in some instances using antisense and ribozyme suppression strategies has led to the reversal of a tumourigenic phenotype, by greatly reducing the expression of an oncogene or cleaving a mutant transcript at the site of the mutation (Carter and Lemoine 1993; Lange et al. 1993; Valera et al. 1994; Dosaka-Akita et al. 1995; Feng et al. 1995; Quattrone et al. 1995). Triple helix approaches have also been investigated for gene suppression. In many cases complete (100%) suppression of gene expression has been difficult to achieve. In some dominant disorders it may be necessary to block expression of the disease allele completely to prevent disease symptoms whereas in others low levels of mutant protein may be tolerated.

But this has the disadvantage that...

Differentiating between the normal and disease alleles and selectively switching off the disease allele using suppression strategies targeted towards the disease mutation in some cases may be difficult - the disease may be the result of a single base change and may be difficult to target specifically. For example, the disease mutation may not occur at a ribozyme cleavage site. Similarly the disease allele may be difficult to target specifically by antisense DNA/RNA or triple helix DNA if there are only small sequence differences between the disease and normal alleles. Moreover some dominant disorders are extremely heterogenous - many different mutations in the same gene give rise to a similar disease phenotype. The development of specific gene therapies for each these is probably not economically viable. To circumvent the dual difficulties associated with specifically targeting a disease allele using the disease mutation and the genetic heterogeneity present in some dominant disorders, a novel strategy for gene therapy for autosomal dominant disease exploiting the degeneracy of the genetic code (the characteristic that amino acids can be coded for by more than a single nucleotide triplet or codon) is described in this invention.

Dependent upon the required use Combination Therapy each to be individually monitored [illegible]...

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STATEMENT OF INVENTION

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According to the present invention there is provided a strategy for suppressing an endogenous gene and replacing at least part of the gene sequence with a nucleic acid sequence which differs from the endogenous gene.

Typically the suppressing agent comprises at least one suppressor from the group comprising antisense nucleic acid, DNA capable of forming triple helix or ribozymes to the endogenous gene.

Suitably, the replacement nucleic acid sequence encodes at least part of a gene product and does not interact with the suppressing agent.

Preferably the replacement nucleic acid sequence comprises amino acid codons which encode at least part of the required gene product, and differ from the gene to be suppressed in at least the third base of at least some of the codons.

The invention therefore uses the degeneracy of the genetic code to ensure that a replacement gene product encoding sequence does not react with a suppressing agent directed to an endogenous gene.

The invention further comprises a medicament comprising a gene suppressing agent and a nucleic acid encoding at least part of a replacement gene product.

The suppressing agent and replacement nucleic acid may be administered separately or together.

The invention further provides a medicament comprising a gene product encoding nucleic acid sequence wherein the sequence differs from an endogenous gene in at least the third base of at least some amino acid codons.

In one embodiment the medicament may comprise a vector.

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DESCRIPTION

(Describe in detail any modifications or alternatives of the invention as are known to you.)

While further modifications and improvements may be made without departing from the scope of this invention, the following is a description of one or more examples of the invention, with reference to the accompanying drawings . . . .

SUMMARY

Proposal: A novel method of gene therapy for treatment of autosomal dominantly inherited disorders and polygenic diseases.

The present invention relates to a strategy for suppression of a disease allele using strategies which do not target the disease mutation specifically but instead can be designed to target a broad range of transcribed sequences of a particular gene. By transcribed sequences is meant sequences transcribed from the disease allele or from both the disease and normal alleles. A particular embodiment of the invention is the use of suppression strategies to suppress the disease allele or to suppress the disease and normal alleles and the use of the wobble hypothesis to enable continued expression of a replacement or reintroduced normal allele. More particularly the strategy circumvents the need for a specific therapy for every mutation in a given gene. In addition the invention allows greater flexibility in choice of target sequences for suppression of a disease allele. The invention also relates to a medicament or medicaments for use in suppressing a gene with a disease allele which is present in a genome of one or more individuals or animals.

Generally the strategy of the present invention will be useful where the gene which is naturally present in the genome of the patient contributes to a disease state. Generally, the gene in question will be mutated, that is, will possess alterations in its nucleotide sequence that affect the function or level of the gene product. For example, the alteration may result in an altered protein product from the wild type gene or altered control of transcription and processing. Inheritance of such a mutation can give rise to a disease phenotype. However, the gene of interest could also be of wild type genotype but contribute to a disease state in another way such that suppression of the gene would alleviate the disease state.

BACKGOUND

Over the past decade we have investigated the molecular pathogenesis of heritable human retinopathies including Retinitis Pigmentosa (RP) and various macular dystrophies; common retinal degenerations with a recognisable genetic component, leading in many instances to severe visual handicap. The goal of our research has been to contribute to an elucidation of the molecular pathologies of human retinal disease. Applying the approach of genetic linkage, in a collaborative study we localised to two x-linked RP genes to the short arm of the X chromosome (Ott et al. 1990). In autosomal dominant forms of RP (adRP) we have localised three genes. The first adRP gene mapped on 3q close to the gene encoding the photoreceptor specific protein rhodopsin (McWilliam et al. 1989). Similarly, we placed an adRP gene on 6p close to the gene encoding the

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photoreceptor specific protein peripherin/RDS (Farrar et al. 1991a,b). A third adRP gene mapped to 7q (Jordan et al. 1993); no known candidate genes for RP reside in this region of 7q. In addition, we placed the disease gene segregating in a Best's macular dystrophy family on 11q close to the region previously shown to be involved in some forms of this dystrophy (Mansergh et al. 1993). Recently, in a collaborative study we placed an autosomal recessive RP gene on 1q (Van Soest et al. 1994). Genetic linkage, in combination with techniques for rapid mutational screening of candidate genes, enabled subsequent identification of causative mutations in the genes encoding rhodopsin and peripherin/RDS proteins. Globally about 100 rhodopsin mutations have now been found in patients with RP or congenital stationary night blindness. Similarly about 40 mutations have been characterised in the peripherin/RDS gene in patients with RP or with various macular dystrophies.

Knowledge of the molecular etiology of some forms of human inherited retinopathies has stimulated us to establish methods to generate animal models for these diseases and to investigate possible methods of therapeutic intervention to alleviate disease symptoms; the primary goal being the development of treatments for human retinal diseases. Surgical procedures enabling the injection of sub-microlitre volumes of fluid intravitreally or sub-retinally in mice have been developed (Dr Paul Kenna). In conjunction with the generation of animal models we are investigating optimal systems for delivery of gene therapies to retinal tissues using both viral and non-viral vectors. Delivering information to retinal tissues, either in gene or drug form, has now become a realistic and exciting possibility. More generally, gene therapies utilising both viral and non-viral delivery systems have been applied to a number of inherited disorders, to cancers and to some infectious disorders. The majority of this work has been undertaken in animal models of human diseases, although some gene therapies have been approved for use in humans. Many gene therapy studies have focused on autosomal recessively inherited disorders such as Cystic Fibrosis, the rationale being that in recessive diseases the introduction and efficient expression of the wild type gene may be sufficient to result in a prevention/amelioration of disease phenotype. In contrast gene therapy for dominant disorders will require the suppression of the dominant disease allele. Notably the majority of mutations characterised to date that cause RP are inherited in an autosomal dominant fashion.

Indeed there are over 1,000 autosomal dominantly inherited disorders in man. In addition in human populations there are many polygenic disorders due to the co-inheritance of a number of genetic components which together give rise to a dominant disease phenotype. Effective gene therapy in dominant disease will require suppression of the disease allele while maintaining the function of the normal allele. Strategies to differentiate between the normal and disease alleles and to selectively switch off the disease allele using antisense DNA/RNA or triple helix DNA targeted towards the disease mutation in many cases may be difficult and in some cases impossible frequently the disease and normal alleles may differ by only a single nucelotide. A further difficulty inhibiting the development of gene therapies is the heterogenous nature of some dominant disorders - many different mutations in the same gene give rise to a similar disease phenotype. Hence, due to these difficulties, to date few gene therapies have been developed for autosomal dominantly inherited disorders.

DESCRIPTION

According to the present invention there is provided a strategy for suppressing expression of a dominant disease allele wherein said strategy comprises providing antisense nucleic acids able to bind to transcripts from both the disease and normal alleles or to bind selectively or partially selectively to transcripts from the disease or normal alleles. Antisense nucleic acids can be DNA or RNA and can be directed to any sequences that are part of the gene that is believed to be involved in a disease phenotype in a given patient. Notably antisense can be directed towards any sequences present in the gene or to

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the sequence around the site of the disease mutation within a gene. For example, antisense nucleic acids can be directed to the sequence around a single nucleotide alteration within a gene which gives rise to the disease. By gene is meant the 5' and 3' controlling sequences associated with the gene and the coding and non-coding sequences of the gene. The binding of the antisense nucleic acids prevents or reduces th functional expression of the disease and normal alleles. If the antisense is directed to the region around the disease mutation suppression of expression of the disease allele may be more efficient than that of the normal allele. The term 'functional expression' means the expression of a gene product able to function in a manner equivalent to or better than a wild-type product.

The invention can employ ribozymes to suppress expression of the disease and normal alleles by cleaving the target transcript at any ribozyme target site within the transcript. The ribozyme can be designed to cleave the transcript from the disease or normal alleles specifically or partially specifically. The invention can employ triple helix DNA to suppress gene expression by triple helix formation directed to the disease and normal alleles or to specifically or partially specifically suppress gene expression of the disease allele by triple helix formation directed to the disease allele.

Antisense nucleic acids, ribozymes and triple helix can be synthesised and directly utilised in therapies or can be incorporated into a vector. Vectors include DNA plasmid vectors or RNA and DNA virus vectors or other vectors for gene delivery. Synthesised gene suppression agents or vectors can be combined with lipids, polymers and other derivatives to aid gene transfer. Oligonucleotides can be synthesised and chemically modified to render them more resistant to cellular nucleases.

According to the present invention there is provided a strategy for suppressing an endogenous gene and introducing a replacement gene or nucleic acid. The replacement nucleic acid will not be recognised or will be recognised less efficiently by the antisense nucleic acid, ribozyme or triple helix (suppression effectors) used for suppression of gene expression. The sequences of the replacement nucleic acid will code for the same amino acids as the wild type endogenous normal gene. The nucleotide sequences targeted by suppression effectors will be altered in the replacement gene. The third nucleotide of a codon may be changed in some cases and can still code for the same amino acid. The degeneracy of the genetic code will be used to alter the replacement gene so that it will not be recognised or will be recognised less efficiently by suppression effectors. Alternatively the sequences of the replacement nucleic acid may code for protein with amino acid changes from the wild type protein which either confer a beneficial effect on the patient or have be shown to have no associated disease pathology. The suppression effectors may be targeted to the region of sequence difference between the endogenous wild type gene and the reintroduced beneficial gene or to any region of the gene. The replacement gene will be masked completely or partially from suppression effectors by exploiting the sequence differences between the wild type gene and the replacement gene or by altering the sequence of the replacement gene to exploit the degeneracy of the genetic code or by a combination of both.

The use of suppression effectors to suppress gene expression together with the use of an altered replacement gene circumvents inherent difficulties with dominant gene therapies. Differentiating between the normal and disease alleles and selectively switching off the disease allele using suppression strategies targeted towards the disease mutation in some cases may be difficult. For example, the disease mutation may not occur at a ribozyme cleavage site (NUX sequence). In such situations binding affinities of ribozyme antisense flanking sequences may possibly be used to differentiate between normal and disease alleles but sometimes may be difficult to apply if, for example, the disease and normal alleles differ by a single nucleotide. Similarly in some cases the disease allele may be difficult to target specifically by antisense DNA/RNA or triple helix DNA if there are only small sequence differences between the disease and normal alleles. In addition, some dominant disorders are heterogenous - multiple mutations in

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the same gene give rise to a similar disease phenotype. The development of specific gene therapies for each these would be extremely costly. To circumvent th difficulties associated with specifically targeting a disease allele using th disease mutation and the genetic heterogeneity present in some dominant disorders, a novel strategy for gene therapy for autosomal dominant disease exploiting the wobble hypothesis is described in this invention.

The strategy described herein has applications to autosomal dominant diseases. Complete (100%) silencing of a disease allele may be difficult to achieve using antisense strategies. However, small quantities of mutant product may be tolerated in some autosomal dominant disorders. In others, a significant reduction in the proportion of mutant to normal product may result in an amelioration in disease symptoms. Hence this strategy may be applied to many autosomal dominantly inherited diseases in man where the molecular basis of the disease has been established. The strategy will enable the same therapy to be used to treat a wide range of different disease mutations within the same gene. Strategies such as this using a 'universal' approach will facilitate the development of treatments for dominant diseases.

This strategy may be applied in gene therapy approaches for biologically important polygenic disorders affecting large proportions of the world's populations such as age related macular degeneration (ARMD), glaucoma, manic depression, cancers having a familial component and indeed many others. Polygenic diseases require the inheritance of more than one mutation (component) to give rise to the disease phenotype. Notably an amelioration in disease symptoms may require the switching off or reduction in the presence of only one of these components. The strategy described here may be applied broadly to possible future interventive therapies in common polygenic diseases to suppress a particular genotype and thereby suppress the disease phenotype.

METHODOLOGIES AND MATERIALS

The suppression approach involves:
Suppression/silencing of the disease and normal alleles or partially selectively suppressing the disease allele of an endogenous gene using antisense RNA/DNA, ribozymes or triple helix DNA targeted to any transcribed sequences from the gene. This enables greater flexibility in the choice of target sequence for suppression. Additionally a replacement normal gene will be supplied with an altered DNA sequence so that it is masked from suppression effectors. The sequence will be altered in third base wobble positions so that the replacement gene codes for a protein which is identical to the wild type protein. In addition using this strategy the same therapy could be applied to the treatment of different disease mutations within a given disease gene.

Genomic and cDNA sequences - sequence degeneracy:
The genomic and cDNA sequences for many genes from various species are available in sequence databases such as Genebank, Embl. For genes where sequence is not available, genomic and cDNA clones can readily be identified and sequenced using standard technologies. For example, genomic sequence for the mouse gene encoding the photoreceptor specific protein rhodopsin is available in DNA sequence databases. In contrast the mouse genomic sequence encoding the photoreceptor specific protein peripherin/RDS is not in sequence databases and has been cloned in both phage and plasmid vectors and sequenced. Nucleotide differences have been observed in the same gene from the same species (Farrar et al. 1991). In many cases nucleotide differences are not associated with a phenotype and have no effect on the gene product as they do not alter an amino acid. All amino acids except tryptophan and methionine are represented by more than one codon. In addition, in some cases nucleotide differences or polymorphisms can result in amino acid changes in the protein product but have no associated disease pathology (Jordan et al. 1993). The degeneracy of the genetic code and the polymorphic nature of some genes can be exploited to facilitate dominant gene

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therapies. Some sequence names and Id's from Genebank are given below. All sequences in databases can be freely accessed. GB_PR:HUMRDS ID:31620205, GB_PR:HUMCHSBS ID: 7d350205 (human peripherin); GB-PR: HUMRASH ID: 98610205 (Human H-Ras); GB-PR: HSNRASE1 ID: e80c0205, GB-PR: HSNRASE2 ID: e90c0205, GB-PR: HSNRASPR ID: caccc205 (Human N-Ras); GB-PR: HUMKRASM ID: d54f0205 (Human K-Ras); GB-PR: HUMLDLR01 ID: 33510205 (LDLR cDNA); GB-PR: HUMLDLRA2 ID:48510205 (LDLR intron4); GB-PR: HUMPKDGEN ID: 875e0205 (Polycystic kidney disease gene)

Degeneracy designs:
Peripherin / RDS gene - Sequences within the human or mouse peripherin/RDS gene can be chosen as targets for suppression effectors (GB_PR:HUMRDS Accession No: M73531). For application in treatment of human disease the human sequences can be used. To facilitate *in vivo* experimental designs mouse sequences can be used. For example, suppression agents targeted towards sequences in peripherin/RDS transcripts but not including codons for tryptophan or methionine can enable the introduction of a replacement peripherin/RDS gene in which every third base of the target sequence is altered from the wild type gene. The replacement peripherin/RDS gene can code for the same amino acids as the wild type gene but cannot be recognised or can less efficiently be recognised by the suppression agent. For example, antisense can be directed towards the sequence coding for amino acids 2-8 of human peripherin/RDS. The sequence of the replacement gene can be altered significantly without changing the amino acids coded for by that sequence.

Normal peripherin/RDS DNA sequence and amino acids

GCG	CTG	CTC	AAA	GTC	AAG	TTT
Ala	**Leu**	**Leu**	**Lys**	**Val**	**Lys**	**Phe**
GCT	CTT	CTT	AAG	GTT	AAA	UUC
GCC	CTC	CTG		GTG		
GCA	CTA	CTA		GTA		
	TTG	TTG				
	TTA	TTA				

Altered DNA sequence coding for the same amino acids

Codon usage can influence the rate of production of a given protein. In many cases codons which are used elsewhere in the protein can be chosen to optimise the effectiveness of the replacement gene.

Ras genes - Mutations in a number of genes, for example, ras genes, have been correlated previously with tumour development or with a predisposition to tumour development (Oyama et al. 1995, Wong et al. 1995). In addition, suppression strategies have in some instances resulted in the reversion of neoplastic phenotypes. Different mutations within ras genes have been observed in DNA from tumour tissues. The described suppression strategy could be exploited to limit the number of therapies required to treat patients. The invention can be used to suppress both mutated and normal alleles and to introduce a replacement gene coding for the wild type protein which is masked from suppression effectors by altering the sequence of the replacement gene at third base wobble positions across the region targeted by the suppression effectors.

Notably the invention described in this patent application could be applied to suppress gene expression of any human gene or gene from other animal species for which there is available sequence information.

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Cloning of cDNA:
Methods of mutagenesis including polymerase chain reaction (PCR) mutagenesis can be used to introduce sequence alterations at wobble positions into cDNA or genomic constructs containing sequences of interest such as peripherin/RDS or ras genes. cDNA and genomic sequences of interest can be cloned into expression vectors which enable expression *in vitro* and in mammalian cell lines such as Cos-7 cells. Constructs can also be used in the generation of transgenic mice for *in vivo* studies.

Generation of antisense and ribozyme constructs / oligonucleotides:
DNA fragments targeted to sequences in the transcribed region of the gene to be suppressed can be cloned in reverse orientation into expression vectors. Constructs can be expressed from a range of promoters including strong general promoters or from tissue specific promoters. When appropriate restriction sites are not available for cloning, primers can be synthesised with restriction sites to facilitate cloning directly or alternatively PCR and subsequent cloning. Likewise ribozymes designed to target specific sites in transcripts can be synthesised and cloned into expression vectors. Ribozyme target sites have been chosen with the aid of computer generated predictions of transcript 2-D structures. Constructs can be used for both *in vitro* and *in vivo* studies. Alternatively, ribozymes, antisense and triple helix DNA can be sythesised using standard technologies. Chemical modifications during or after synthesis can be used to increase the stability of suppression effectors. Chemically synthesised ribozymes, anitsense and triple helix DNA can be used *in vitro* and *in vivo* and efficiency of gene silencing assayed.

In vitro expression:
The efficiency of antisense DNA/RNAs, ribozymes and triple helix DNA at suppressing gene expression of disease and normal alleles can be assessed using *in vitro* transcription and translation in test tubes (for example, Ribomax kits) and *in mammalian cells* (such as Cos-7 cells). The specificity and efficiency of suppression effectors at targeting transcripts from a disease allele when the target site includes the site of the disease mutation can also be assessed *in vitro*. Effects of suppression effectors on gene expression can be assayed by gel electrophoresis, northern blotting, quantitative reverse transcription (rt)-PCR and western blotting. Suppression agents (antisense, ribozymes, triple helix) can be synthesised (with/without chemical modifications to render oligonucleotides more resistant to cellular nucleases) and used for *in vitro* studies. Alternatively suppression effectors can be expressed from vectors *in vitro*. Suppression effectors can be administered directly or in lipid or polymer complexes (or in complexes with other derivatives) for *in vitro* studies.

In vivo expression:
Gene suppression can be assayed *in vivo* by generating appropriate transgenic animals. Transgenic mice with suppression constructs containing antisense and ribozymes can be generated using standard transgenic technology. In addition, transgenic mice carrying a replacement gene with an altered nucleotide sequence can be generated. Constructs generated for *in vitro* studies can also be used in the creation of transgenic mice for *in vivo* studies. Transgenic animals with various combinations of transgenes expressing gene suppression effectors and replacement genes can be mated. Subsequent effect(s) of suppression effectors on gene expression can be assayed by gel electrophoresis, northern blotting, rt-PCR and western blotting. In parallel synthesised antisense, ribozymes and triple helix (with/without chemical modifications to render oligonucleotides more resistant to cellular nucleases) will be administered directly or in lipid or polymer complexes (or in mixes with other derivatives) for *in vivo* studies in mice.

Somatic gene transfer - gene delivery:
Gene therapies to suppress expression from dominantly inherited mutations or indeed components giving rise to polygenic disorders may require delivery of therapies to target somatic tissue, efficient, safe and persistent expression of the therapeutic gene or sequence. The invention could be delivered as naked DNA, in non-viral vectors such as

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DNA: lipid complexes, (cationic lipid and non-cationic lipids) DNA: polymer complexes (cationic polymers such as dendrimers, neutral polymers) or in combination with other non-viral vectors. An alternative approach for d livery of the invention is the use of recombinant viruses, either integrating or non-integrating viruses *inter alia* recombinant adenovirus, adeno associated virus, herpes simplex virus, and retroviruses such as moloney murine leukaemia virus.

Conclusions:
The invention outlined here aims to provide a strategy to silence dominant mutations using antisense sequences, ribozymes and/or triple helix DNAs that are directed towards a wide choice of sequences within a gene to be silenced rather than a specific disease mutation. The invention allows great flexibility in choice of target sequence for suppression. In some cases the sequence across the disease mutation can be chosen as the target sequence for suppression. The approach results in either the suppression of expression of both the disease and normal alleles or in some cases the disease or normal allele may be specifically or partially specifically suppressed. The normal gene is reintroduced with an altered nucleotide sequence around the target site so that transcripts from this replacement gene escape suppression. The sequence alterations are designed in third base positions so that the coded amino acids remain unaltered. The rationale for the strategy is that in many cases it may be advantageous to suppress expression of mutations which lead to a disease phenotype or predispose for it. This novel approach provides a method for suppression of dominantly inherited mutations which overcomes the need to suppress specific mutations in a given gene. In contrast therapies targeted to single disease mutations will be useful only in the treatment of patients with the specific disease mutation. In addition targeting the disease allele specifically may be difficult to achieve. The invention provides a method which enables greater flexibility in choice of target sequence to silence a disease allele. The target sequences can be chosen in any part of the coding sequence. The invention will have many applications in gene suppression of dominant mutations and mutations predisposing to a disease pathology in humans and other animal species.

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5A

ADVANTAGES

The advantages of the invention and/or the ways in which the disadvantages of previously known arrangements are overcome, include.....

The present invention relates to a 'universal' approach for suppression of dominantly acting mutations. In many cases dominant mutations may be the result of single nucleotide changes. Hence in some situations there may be great difficulty in distinguishing between the normal and disease alleles using suppression effectors such as antisense or ribozymes. Moreover in the case of ribozymes the sequence around the mutation may not occurr at a ribozyme cleavage site or may not be accessible due to the RNA 2-D structure. The present invention circumvents these difficulties as the target sequence for suppression can be designed anywhere in the coding region of the gene thereby providing much greater flexibility in choice of target sequence for suppression and in turn optimising the likelihood of obtaining optimal suppression.

In addition both the normal and disease genes are suppressed converting the dominant disease into effectively a recessive disease where it is then necessary only to supply a replacement normal gene. The replacement normal gene has an altered nucleotide sequence exploiting the wobble hypothesis or the degeneracy of the genetic code. Hence transcripts from the replacement gene have an altered sequence around the suppression target site and are masked from suppression effectors. Notably the replacement gene codes for the same amino acids as the wild type gene.

A particular advantage of this invention is that unlike other suppression strategies for dominant diseases which typically target the disease mutations specifically the present strategy is not disease mutation specific. Any mutation within a given gene can be suppressed using the same strategy or therapy. This is particularly powerful in situations where tens or hundreds of mutations in the same gene give rise to a disease pathology.

(ATTACH ALL RELEVANT DRAWINGS AND/OR PHOTOGRAPHS.)

Should extra pages be required see Continuation Sheet, each to be individually numbered 5B, 5C ...